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Cancer Diagnostics and Molecular Pathology

Genomic Signature of Driver Genes Identified by Target Next-Generation Sequencing in Chinese Non-Small Cell Lung Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Non-small cell lung cancer • Next-generation sequencing • Molecular genomic profile

Abstract

Background. Non-small cell lung cancer (NSCLC) is one of the most common human malignancies and the leading cause of cancer-related death. Over the past few decades, genomic alterations of cancer driver genes have been identified in NSCLC, and molecular testing and targeted therapies have become standard care for lung cancer patients. Here we studied the unique genomic profile of driver genes in Chinese patients with NSCLC by next-generation sequencing (NGS) assay.

Materials and Methods. A total of 1,200 Chinese patients with NSCLC were enrolled in this study. The median age was 60 years (range: 26–89), and 83% cases were adenocarcinoma. NGS-based genomic profiling of major lung cancer-related genes was performed on formalin-fixed paraffin-embedded tumor samples and matched blood.

Results. Approximately 73.9% of patients with NSCLC harbored at least one actionable alteration recommended by the National Comprehensive Cancer Network guideline, including epidermal growth factor receptor (*EGFR*), *ALK*, *ERBB2*, *MET*, *BRAF*, *RET*, and *ROS1*. Twenty-seven patients (2.2%) harbored inherited germline mutations of cancer susceptibility genes. The frequencies of *EGFR* genomic alterations (both mutations and amplification) and *ALK* rearrangement were identified as 50.1% and 7.8% in Chinese NSCLC populations, respectively, and significantly higher than the Western population. Fifty-six distinct uncommon *EGFR* mutations other than L858R, exon19del, exon20ins, or T790M were identified in 18.9% of patients with *EGFR*-mutant NSCLC. About 7.4% of patients harbored both sensitizing and uncommon mutations, and 11.6% of patients harbored only uncommon *EGFR* mutations. The uncommon *EGFR* mutations more frequently combined with the genomic alterations of *ALK*, *CDKN2A*, *NTRK3*, *TSC2*, and *KRAS*. In patients <40 years of age, the *ALK*-positive percentage was up to 28.2%. Moreover, 3.2% of *ALK*-positive patients harbored multi ALK rearrangements, and seven new partner genes were identified.

Conclusion. More unique features of cancer driver genes in Chinese NSCLC were identified by next-generation sequencing. These findings highlighted that NGS technology is more feasible and necessary than other molecular testing methods, and suggested that the special strategies are needed for drug development and targeted therapy for Chinese patients with NSCLC. ***The Oncologist*** 2019;24:e1070–e1081

Implications for Practice: Molecular targeted therapy is now the standard first-line treatment for patients with advanced non-small cell lung cancer (NSCLC). Samples of 1,200 Chinese patients with NSCLC were analyzed through next-generation sequencing to characterize the unique feature of uncommon EGFR mutations and ALK fusion. The results showed that 7.4% of EGFR-mutant patients harbored both sensitizing and uncommon mutations and 11.6% harbored only uncommon mutations. Uncommon EGFR mutations more frequently combined with the genomic alterations of ALK, CDKN2A, NTRK3, TSC2, and KRAS. ALK fusion was more common in younger patients, and the frequency decreased monotonically with age. 3.2% of ALK-positive patients harbored multi ALK rearrangement, and seven new partner genes were identified.

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Introduction

Lung cancer is the most fatal malignancy in China [1, 2]. An estimated 733,300 newly diagnosed lung cancer cases and 610,200 lung cancer-related deaths were reported in China in 2015, and traditional surgery and radio-chemotherapy are of limited help to improve the survival of the patients [3]. Following the discovery of cancer driver genes and the development of next-generation sequencing (NGS) technology, genomic alterations have been integrated as part of the standard diagnostic procedure, and several targeted drugs targeting the driver gene mutations have been applied in clinic and achieved certain therapeutic effects [4–7].

The major type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for approximately 85% of all cases [8]. Epidermal growth factor receptor (*EGFR*) gene mutations can be detected in approximately 10%–20% in Western patients with advanced NSCLC, but as high as 40%–50% in the nonsmoking Asian population [9–11]. In 2003, *EGFR* alterations, including point mutations and short insertions/deletions (indels), were identified as the first druggable mutations in NSCLC and proved to be the most potent predictive biomarker for EGFR tyrosine kinase inhibitors (TKIs) [7]. Thus far, the U.S. Food and Drug Administration (FDA) has approved several EGFR TKIs for NSCLC. Additionally, the Chinese National Medical Products Administration has approved the first-generation EGFR-TKI, icotinib, for first-line treatment of patients with metastatic NSCLC with sensitizing *EGFR* mutations [12–16]. In NSCLC, the most common sensitizing *EGFR* alterations are deletions in exon 19 (approximately 45% of patients with *EGFR* alterations) and L858R mutation in exon 21 (approximately 40%) [10]. But more than half of patients with disease progression after response to initial EGFR-TKI treatment harbor an acquired TKI-resistant *EGFR* T790 M mutation [17–19]. The third-generation EGFR TKI, osimertinib, developed to target *EGFR* T790 M and other *EGFR* sensitizing mutations, has been approved by the FDA for the first-line treatment of patients with metastatic NSCLC with *EGFR* exon 19del or L858R and the treatment of patients with metastatic *EGFR* T790 M mutation-positive NSCLC whose disease has progressed on or after EGFR-TKI therapy [20]. In addition, first-generation EGFR TKI is effective in treating NSCLC with common *EGFR* sensitizing mutations (exon 19deletion and L858R), but it is less effective in treating NSCLC with uncommon *EGFR* mutations, such as G719X or L861Q [21]. The average progression-free survival (PFS) of patients with nonconventional *EGFR*-carrying lung adenocarcinoma treated with gefitinib is only 7.7 months, significantly ($p < .001$) shorter than the 11.4 months of patients with common *EGFR*-carrying lung adenocarcinoma also treated with gefitinib [22]. However, according to the combined analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6, the second-generation TKI afatinib was active in patients with NSCLC who harbored certain types of uncommon *EGFR* mutations, especially G719, L861Q, and S768I [23].

Gene fusion or rearrangement, especially kinase gene fusion, is another typical genomic variation in lung cancer. The most common rearrangement genes in NSCLC are *ALK* [24, 25], *ROS1* [26], and *RET* [27], representing 2%–7%, 1%–2% and 1%–2% of patients, respectively [25–30]. The echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* constitutes a major subset of *ALK* fusions. Patients with NSCLC with *EML4-ALK* likely are of adenocarcinoma histology, never/light smokers, men, and younger, and they do not benefit from EGFR TKIs [31]. Similarly, *ROS1* rearrangements occur more frequently in younger women with adenocarcinoma histology and never smokers [26, 32]. Patients with *ALK* and *ROS1* rearrangements are predicted to benefit from TKIs, such as crizotinib [33–36]. The National Comprehensive Cancer Network (NCCN) Guideline for NSCLC also recommends cabozantinib and vandetanib for patients with *RET* rearrangements [37–39].

In the present study, genomic alterations of driver genes were assessed in a Chinese NSCLC cohort of 1,200 patients by NGS in the College of American Pathologists (CAP)-accredited lab. We described the basic profile of the patient's gene mutations, particularly those that are targeted therapy available, and compared them with The Cancer Genome Atlas (TCGA) and published data (mainly Western people in Europe and the U.S.). Germline mutations can be passed on to offspring to form hereditary cancers. Thus, we also analyzed the germline mutation in Chinese patients with NSCLC. Finally, we analyzed the unique pattern of *EGFR* variations and *ALK* rearrangements, the most common and important driver genes in Chinese NSCLC populations.

Materials and Methods

Samples Source and Ethic Data

In total, 1,200 formalin-fixed, paraffin-embedded (FFPE) tumor samples and matched blood samples were collected and prepared according to standard procedures. All the cases were diagnosed as NSCLC according to World Health Organization criteria based on hematoxylin and eosin staining reviewed by experienced pathologists. All subjects involved in this study were clearly informed, understood the contents of the study, and agreed to publish the results of the study. All patients had signed an informed consent.

Next-Generation Sequencing

Genomic profiling was performed in the laboratory at OrigiMed (Shanghai, China), a CAP-accredited lab. At least 50 ng of cancer tissue DNA was extracted from each 40 mm FFPE tumor sample using a DNA Extraction Kit (QIAamp DNA FFPE Tissue Kit; Qiagen, Hilden, Germany) according to manufacturer's protocols. All the coding exons of 37 key cancer-related genes and selected introns of 8 genes (supplemental online Table 1) were captured by the custom hybridization capture panel. In addition, the probe density was increased to ensure high efficiency of capture in the conservatively low read depths region. Libraries were each diluted to 1.05 nM and then sequenced with a mean coverage of 900× for FFPE samples (minimum 700×) and 300× for matched blood samples on an Illumina NextSeq-500 Platform (Illumina Incorporated, San Diego, CA).

Table 1. Clinical characteristics of 1,200 Chinese patients with NSCLC

Characteristic		*n* (%)
Age	Mean (SD)	59.8 (10.6)
	Median (range)	60 (26–89)
Sex	Male	676 (56.4)
	Female	524 (43.6)
Histology	Adenocarcinoma	1001 (83.4)
	Squamous cell lung cancer	155 (12.9)
	Large cell lung cancer	9 (0.8)
	Mixed lung cancer	24 (2.0)
	NSCLC without histology subtype	11 (0.9)
Stage	I	289 (24.1)
	II	132 (11.0)
	III	162 (13.5)
	IV	514 (42.8)
	Unknown[a]	58 (4.8)
	Not available	42 (3.5)

[a]Patients with unknown clinical stage indicated that the clinical stages were not clarified according to the information from physicians.
Abbreviation: NSCLC, non-small cell lung cancer.

Bioinformatics Analysis

Genomic alterations, including single nucleotide variants (SNVs), short and long insertions/deletions (indels), copy number variations (CNVs), and gene rearrangements, were subjected to advanced analysis. First, reads were aligned to human genome reference sequence (hg19) by Burrows-Wheeler Aligner, and polymerase chain reaction (PCR) duplicates were removed using Picard. Second, SNVs and short indels were identified by MuTect after quality recalibration and realignment using Genome Analysis Toolkit (GATK) and in-house pipeline. Short indels were then calibrated using the results from Pindel. Read depths were normalized within target regions by Exome Copy number Alterations/Variations annotATOR (EXCATOR). The log-ratio per region of each gene was calculated, and customized algorithms were used to detect copy number changes. Tumor cellularity was estimated by allele frequencies of sequenced SNPs. A customized algorithm was developed to detect gene rearrangements and long indels.

Reliable somatic alterations were detected in the raw data by comparison with matched blood control samples. At minimum, five reads and minimum variant allele frequency of 1% were required to support alternative calling. For CNVs, focal amplifications were characterized as genes with thresholds ≥4 copies for amplification and 0 copies for homozygous deletions. For the calling of gene rearrangements, aligned reads with abnormal insert size of over 2,000 or zero bp were collected and used as discordant reads. Next, the discordant reads with the distance less than 500 bp formed clusters that were further assembled to identify potential rearrangement breakpoints. The breakpoints were reconfirmed by the BLAST-like alignment tool and the resulted chimeric gene candidates were annotated. Clinically relevant genomic alterations were further marked as druggable genomic alterations in current treatments or clinical trials. IBM SPSS Statistics (Version 20.0; IBM Corp, Armonk, NY) was used for statistical analysis. For all test, $p < .05$ was defined as statistically significant.

Results

Demographic and Clinicopathological Data of the Patients

The demographics and clinicopathological data of patients in the cohort are summarized in Table 1. The median age of patients at the time of sampling was approximately 60 years (range: 26–89 years), and males were moderately overrepresented compared with females (56% of patients were male). Although there were approximately equal numbers of male and female patients younger than 60, male patients were significantly overrepresented in the ≥60 age group (61% vs. 39%, $p = .0007$). Regarding histological subtypes, adenocarcinoma was the most common subtype (83%) and large cell carcinoma was the least common subtype (0.8%). In addition, .9% of the cases were of uncertain subtypes. Patients were classified into main clinical stages (I–IV) according to both pathology and medical history following the American Journal of Critical Care Cancer Staging Manual (version eight; Table 1). Patients in different stages had similar age distributions (the median was approximately 60 years of age). Males were particularly prevalent in the older (≥60 years of age) and late-stage (III–IV) groups.

Common and Uncommon EGFR Alterations in Chinese Patients with NSCLC

In total, 47.6% of the 1,200 patients were identified to have *EGFR* mutations, which excluded amplification of *EGFR*. 88.4% of *EGFR*-mutant patients harbored hotspots, including L858R (33.4%), exon 19 deletion (36.7%), T790M (5.5%), and exon 20 insertions (3.8%). Figure 1A showed the profile of co-occurring *EGFR* genomic alterations. The majority (57.2%) possessed only mutations (SNV and indel), and a decent number had both mutations and amplifications (22.6%); 4.8% had only amplifications (4.3%) and structure variations (0.5%) including kinase domain duplication (KDD) and *EGFR-RAD51* rearrangement. The percentage of patients with NSCLC with multiple *EGFR* mutations was 15.3% of the patients with *EGFR*-altered NSCLC. Moreover, uncommon mutations accounted for 20.6% of *EGFR* mutations (Fig. 1B).

Uncommon EGFR mutations were defined as the mutations other than L858R, exon 19del, exon 20ins, and T790M. In our study, 154 uncommon *EGFR* mutations in 108 patients, which accounted for 18.9% of *EGFR*-mutant patients, were identified. Two previous studies reported that approximately 6.5% of uncommon *EGFR*-mutant patients were detected in Chinese patients with NSCLC by Sanger sequencing or amplification refractory mutation system (ARMS) technologies [40, 41]. Uncommon *EGFR* mutations that co-occurred with sensitizing mutations were identified in 7.4% of *EGFR*-mutant patients, and 11.6% of *EGFR*-mutant patients harbored only uncommon mutations. Among all the uncommon mutations, 12.3% occurred in the extracellular domain, 1.9% were in the transmembrane domain, 5.8% were in the autophosphorylation domain, 76.6% were in the kinase domain of exon 18–21, and 3.2% were structure variations of KDD and rearrangements

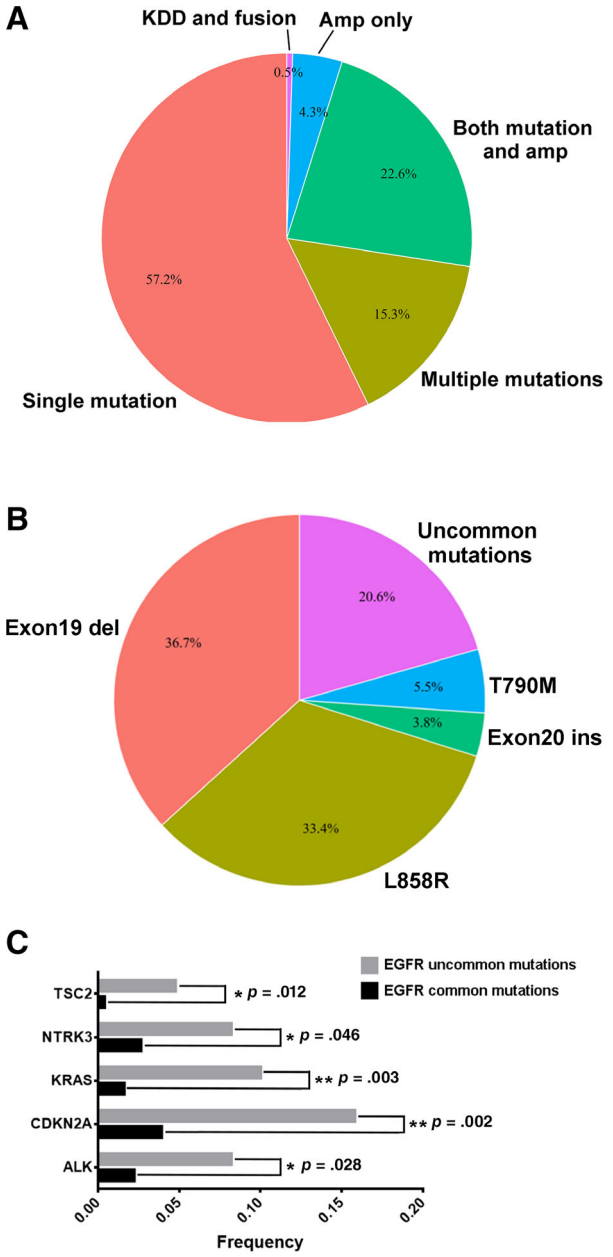

Figure 1. Distribution of EGFR mutations in Chinese cohort. Profiling of overlapping EGFR alteration types **(A)** and mutation spectra among Chinese patients with EGFR-mutant non-small cell lung carcinoma **(B)**. Co-occurring of EGFR common and uncommon mutations with other genomic alterations were analyzed **(C)**. *Indicates $p < .05$; **Indicates $p < .01$. Abbreviations: EGFR, epidermal growth factor receptor; KDD, kinase domain duplication.

(Table 2). A total of 35.7% of the uncommon mutations occurred in exon 18. G719X, S768I, L861X, and E709X were the most frequently identified recurrent uncommon mutations, which accounted for 21.4%, 10.4%, 8.4%, and 7.8% of uncommon *EGFR* mutations, respectively.

EGFR alterations occurred more frequently in females (in contrast to all other driver gene mutations) and often in combination with *TP53* mutations; patients with *EGFR*-mutated squamous carcinoma were mainly males (82%) and always carried *TP53* comutations. The age, sex, and histological type characteristics of patients with NSCLC with only uncommon *EGFR* mutations or common mutations are summarized in Table 3. The incidence of uncommon *EGFR* mutations only (4.5%) was much higher than that of common *EGFR* mutations (0.6%) in lung squamous cancer.

Uncommon *EGFR* mutations were found to occur combined with genomic alterations of *CDKN2A* ($p = .002$), *KRAS* ($p = .003$), *TSC2* ($p = .012$), *ALK* ($p = .028$), and *NTRK3* ($p = .046$) more frequently (Fig. 1C). Further analysis of co-occurring *EGFR* mutations and kinase receptor fusions revealed that six Chinese patients with *EGFR*-mutant NSCLC harbored both *EGFR* mutations and known druggable kinase receptor rearrangements, including *ROS1*, *RET*, and *NTRK*. Three of the six patients received EGFR TKI as the standard treatment. One patient achieved partial response for 10 months, and two patients achieved stable disease for 5 and 4 months.

ALK Rearrangement in Chinese Patients with NSCLC

In the Chinese cohort, we identified 97 *ALK* rearrangements in 94 patients, and there were 3 patients (3.2%) who harbored multi *ALK* rearrangements (Table 4). Younger patients were more likely to harbor *ALK* rearrangements (Fig. 2). In patients aged <40 years, the *ALK*-positive percentage was up to 28.2%, and the percentage decreased along with age to 3.1% in patients aged 70 years and above. The number of Chinese patients with NSCLC with age <40 was relatively low ($n = 39$). One in three of such younger patients harbored *ALK* rearrangements and may potentially benefit from targeted ALK-inhibitor therapies. In terms of gender, the *ALK*-positive percentages in male and female patients with NSCLC were 7.2% and 8.6%, respectively, but the difference was not statistically significant. Analysis of the partner genes of *ALK* rearrangement identified in Chinese patients with NSCLC revealed that *EML4-ALK* contributed to 88.7% of *ALK* rearrangement, and seven new partners were identified in our study including *CDK15-ALK*, *EML6-ALK*, *FBXO11-ALK*, *CAMKMT-ALK*, *YAP1-ALK*, *MEMO1-ALK*, and *LCLAT1-ALK*. The frequency of *EML4-ALK* subtypes is shown in Figure 3. The most common subtypes of *EML4-ALK* were E6:E20 (variant 3; 36.9% of all *EML4-ALK* cases) and E13:E20 (variant 1; 31.0% of all *EML4-ALK* cases), whereas E20:E20 (variant 2) accounted for 14.3% of all *EML4-ALK* cases. Interestingly, E6-E20 was enriched in male patients (18 vs. 13), whereas E13-E20 was enriched in female patients (15 vs. 11). Other uncommon subtypes of *EML4-ALK* in the Chinese cohort were identified (Table 5).

Profiling of Genomic Alterations in 1,200 Chinese Patients with NSCLC

Overall, the average mutations per cancer sample were 2.5 and 2.3 in the samples from male and female patients, respectively. The incidence of genomic alteration is summarized in Figure 4. For each gene, the mutational profile (frequency and composition) in the Chinese cohort (left column) was compared with that in the corresponding TCGA data sets (right column) [42], which were mostly based on Western populations and whole exome sequencing. The genomic alteration incidences of most of the 37 lung cancer-related genes showed statistic differences between Chinese and Western not only in total NSCLC populations but also the subtypes of adenocarcinoma and squamous cell carcinoma. Different genes had

Table 2. Uncommon EGFR mutations identified in Chinese patients with non-small cell lung cancer

EGFR exons/domains	Mutation types	Mutations in LUAD	Mutations in LUSC	Mutations in mixed or other subtypes	Percentage of EGFR uncommon mutations
Extracellular domain	L62R	3			12.3%
	E84V	1			
	R108K/S	5			
	G131R	1			
	S229C	1			
	G239A	1			
	N280K	1			
	S286C	1			
	A289V	1			
	G312W	1			
	D587E	1			
	G598V	1	1		
Transmembrane domain	S645C		1		1.9%
	V651 L	1			
	R680W		1		
Exon 18(nucleotide-binding loop)	Q701L	1			35.7%
	L703I/V	2			
	I706T	3			
	E709A/K	12			
	K714 N			1	
	L718 V	1			
	G719A/C/S	33			
	T725M	2			
Exon 19	I740_K745dup	1			4.5%
	I744M	1			
	L747P	1			
	T751P	1			
	P753L	1			
	A755D	1			
	I759N	1			
Exon 20	S768I	16			16.2%
	V769 L	3			
	H773L	1			
	V774 M	1			
	R776H	1			
	G779C	2			
	G810C	1			
Exon 21(activation loop)	L833 V	4			20.1%
	V834 L	4	1		
	H835L	3			
	L838 V	2			
	V843I	1			
	L861 M/Q/R	13			
	A871E/G	3			

(continued)

Table 2. (continued)

EGFR exons/domains	Mutation types	Mutations in LUAD	Mutations in LUSC	Mutations in mixed or other subtypes	Percentage of EGFR uncommon mutations
Autophosphorylation domain	E922K	1			5.8%
	G930E	1			
	M945I	1			
	I981F			1	
	D1014Y	1			
	G1022S	1			
	Y1069C	1			
	E1137K	1			
	A1201S	1			
Structure variations	EGFR-KDD	3			3.2%
	EGFR-RAD51	1			
	vIII (exon2-7del)		1		

Abbreviations: EGFR, epidermal growth factor receptor; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

Table 3. Comparison of characteristics between common and uncommon EGFR mutations in Chinese NSCLC patients

Characteristics	Uncommon only (n = 66)	Common (n = 505)	p value
Age, years			
Median (range)	62.5 (36–88)	59 (27–89)	.333
Sex, n (%)			
Male	33 (50.0)	184 (36.4)	.033[a]
Female	33 (50.0)	321 (63.6)	
Histological type, n (%)			
Adenocarcinoma	62 (93.9)	490 (97.0)	.188
Squamous	3 (4.5)	3 (0.6)	.003[b]
Other NSCLC	1 (1.5)	12 (2.4)	.659

[a]This p value means that the sex distribution of the people carrying common EGFR mutation is different, and the proportion of men is significantly lower than that of women.
[b]This p value represents the difference of detection rate between uncommon EGFR mutation and the common EGFR mutation in squamous lung cancer.
Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

markedly distinct composition of alteration types. For example, *TP53* and *EGFR* were the most frequently mutated genes, and both had SNV as the major alteration type. Of particular interest were common actionable genomic alterations (involving *EGFR*, *KRAS*, *ALK*, *ROS1*, *RET*, *MET*, *BRAF*, *PIK3CA*, *PTEN*, and *NTRK1/3*) in the Chinese NSCLC cohort. The most frequent genomic alterations were *EGFR* (50.1% of cases harbored at least one *EGFR* mutation or amplification), *KRAS* (12.3% of cases harbored *KRAS* mutations or amplification), and *ALK* rearrangement (7.8%). Other frequent genomic alterations were as follows: *PIK3CA* (12.0% both for mutations or amplification), *ERBB2* alterations (6.3%; 4.3% mutations and 2.4% amplifications), *PTEN* (4.0% both for mutations or deletion), *BRAF* (4.4%), *MET* alterations (3.4%; 3.0% amplifications and 0.4% exon 14 skipping mutations), *RET* rearrangement (2.3%), and *ROS1* rearrangement (1.3%).

Approximately 73.9% of cases harbored at least one genomic alteration, including SNV, short indel, long indel, CNV, and gene rearrangement, in druggable genes recommended by the NCCN guideline, including *EGFR*, *ALK*, *ERBB2*, *MET*, *BRAF*, *RET*, and *ROS1*, which showed ethnic differences between Chinese and Western NSCLC populations identified by target sequencing assay of Foundation One as well [9] (Table 6). Gene rearrangements in kinase genes including *ALK*, *BRAF*, *EGFR*, *NTRK1/3*, *MET*, *ERBB2*, *PDGFRA*, *RET*, and *ROS1* occurred in 13.2% of Chinese patients with NSCLC. *MET* exon 14 skipping was detected at 0.4% compared with that commonly previously reported at 3%, which may represent a difference in Chinese versus Western populations.

In addition to the common genes mentioned above, the incidences of some other less common genes were also compared in our study. Potential actionable tumor suppressor genes such as *CDKN2A/B*, *BRCA1/2*, *PTEN*, and *TSC1/2* showed statistic differences in prevalence between Chinese and Western populations with NSCLC. Considering the histological subtypes of NSCLC, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) displayed different patterns of racial diversity. The frequency of cell cycle-related genes *CDKN2A* was much lower in both LUAD (5.1% vs. 21.5%, $p < .0001$) and LUSC (23.2% vs. 43.6%, $p = .0024$), and genomic alterations of *BRCA2* occurred more in the Chinese population than the Western cohort in both LUAD (2.9% vs. 0.0%, $p < .0001$) and LUSC (3.2% vs. 0.0%, $p = .0008$). However, the prevalence of *TSC1/2* and *BRCA1* only showed difference in LUAD and not in LUSC.

Germline Mutation of Cancer Susceptibility Genes in Chinese Patients with NSCLC

In the present study, 27 patients (2.2%) with NSCLC harbored inherited germline mutations of cancer susceptibility genes (Fig. 5). Of those, the most frequently occurring were BRCAness genes (14 patients, 51.9%). BRCAness is a term used for genes whose mutations in cancer cells behave similarly to tumors with *BRCA1* or *BRCA2* mutations. Loss of function germline mutations of BRCAness genes included mutations on *BRCA1/2* (five

Table 4. Patient characteristics with dual ALK rearrangements

Patients	Histological subtype	Sex	Age, years	Stage	ALK rearrangement
P1	Adenocarcinoma	Female	54	IV	EML4 exon6-ALK exon20 CDK15 exon10-ALK exon19
P2	Adenocarcinoma	Male	56	IV	EML6 exon1-ALK exon20 ALK exon19-FBXO11 exon2[a]
P3	Adenocarcinoma	Male	44	IIB	EML4 exon6-ALK exon20 ALK exon19-MEMO1 exon8[b]

[a]Predicted the existence of FBXO11 exon1-ALK exon20.
[b]Predicted the existence of MEMO1 exon7-ALK exon20.

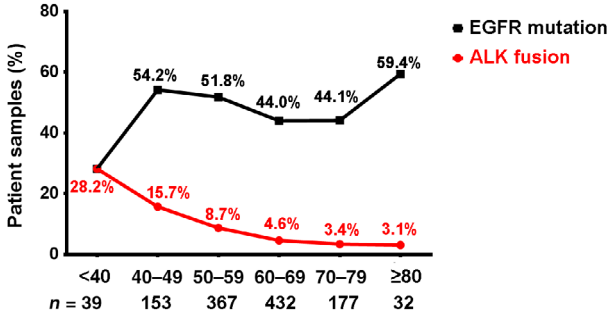

Figure 2. The incidence of ALK fusion and EGFR mutations in Chinese NSCLC according to different age groups. Red curve represents the frequency of patients with ALK fusion in each age group. Black curve represents the frequency of patients with EGFR mutations in each age group.

patients), *FANCA* (four patients), *ATM* (one patient), *BARD1* (one patient), *BRIP1* (one patient), *CHEK2* (one patient), and *PALB2* (one patient). In addition, seven patients harbored inactivating germline mutations of mismatch repair (MRR) genes, including *MLH1* (one patient), *MSH2* (one patient), *MSH6* (one patient), *PMS2* (three patients), and *EPCAM* (one patient). The following two *EGFR* germline mutations were also identified in the Chinese cohort: a T790 M mutation in a 27-year-old male adenosquamous subtype patient and a G724S mutation in a 57-year-old female adenocarcinoma subtype patient; both patients have a family history of lung cancer. To the best of our knowledge, this is the first report of the germline *EGFR* G724S mutation. Previous studies have suggested that *EGFR* G724S is oncogenic [43, 44] and that the *EGFR* T790M germline mutation is associated with inherited NSCLC [45–47]. Other pathogenic germline mutations were identified in *APC*, *TSC1*, *SDHC*, and *SPINK1*. In addition, patients with pathogenic germline mutations of cancer susceptibility genes were 7 years younger than patients without those mutations (54 vs. 61 years of age).

Discussion

Most patients with NSCLC are diagnosed at the middle to late stage despite recent advances of early detection screening for lung cancer. Fortunately, NGS technology now is in wide use to identify cancer gene mutations and provide molecular basis for appropriate targeted therapy in clinical precision medicine. In this NGS-based study of Chinese patients with NSCLC, a comprehensive profile of genomic alterations was constructed. In gene mutations of *EGFR*, *KRAS*, and *ALK*, Chinese patients with NSCLC were different from the Western population, as the former had a much higher frequency (47.6% vs. 20.0%) of *EGFR* mutations and *ALK* rearrangements (7.8% vs. 4.1%) but a significantly lower frequency (10.8% vs. 32.0%) of *KRAS* mutation than the latter [9], which was consistent with previous reports [48–50]. Nearly three quarters of Chinese patients with NSCLC harbored at least one druggable genomic alteration of driver genes recommended by the NCCN guideline, revealing the different clinical characteristics of Chinese patients. However, the TCGA data sets were generated by a different technological platform (Affymetrix SNP6.0 arrays) and bioinformatics suite (e.g., GISTIC for CNV calling and RNAseq for fusion calling).

The patients with NSCLC with *EGFR* mutation may benefit from treatment using EGFR TKIs. In this study, the two most common *EGFR* mutations identified in Chinese patients with NSCLC were exon 19 deletion (36.7%) and L858R mutation (33.4%), which are sensitive to EGFR TKIs, and this was consistent with the findings of the study published with Western people as the main subjects [51]. The exon 20 insertions, which were mostly insensitive to EGFR TKIs [52, 53], amounted to 3.8% of *EGFR* mutations, and the most common resistant mutation, T790M [54, 55], accounted for 5.5% of EGFR mutations. For those 5.5% of patients with primary *EGFR* T790M mutation, which suggests primary resistance, selecting one of the third-generation EGFR TKIs as first-line therapy is advisable. NGS is currently the most effective way to detect *EGFR* gene alterations [51]. By increasing the depth of sequencing, some less common and low-frequency mutations may be discovered. In this study, the detection rate of uncommon *EGFR* mutation only was 11.6% of *EGFR* mutant patients, which was higher than about 6.5% from previous studies. This finding may be related to the advantage of the whole exon coverage of *EGFR* of our NGS assay and the increase of sequencing depth. We used 900× depth sequencing and found a total of 56 different types of uncommon *EGFR* mutations, which could be observed in the extracellular domain (12.3%), transmembrane domain (1.9%), exon 18 (nucleotide-binding loop, 35.7%), exon 19 (4.5%), exon 20 (16.2%), exon 21 (activation loop, 20.1%), autophosphorylation domain (5.8%), and structure variations (3.2%). Some uncommon *EGFR* mutations with high incidence (more than 10 cases) included L861 M/Q/R, G719A/C/S, S768I, and E709A/K, which have been described previously [56, 57]. L861Q, G719X, and S768I were *EGFR* mutations with weak activities; the median PFS of patients who received EGFR TKIs as first-line treatment was 7.6 months, 8.2 months, and 3.4 months, respectively, which was significantly shorter than that in patients with common *EGFR* mutations (exon 19 deletion and L858R) [56]. The E709A mutation is located in *EGFR* exon 18 and often combined with other forms of *EGFR*

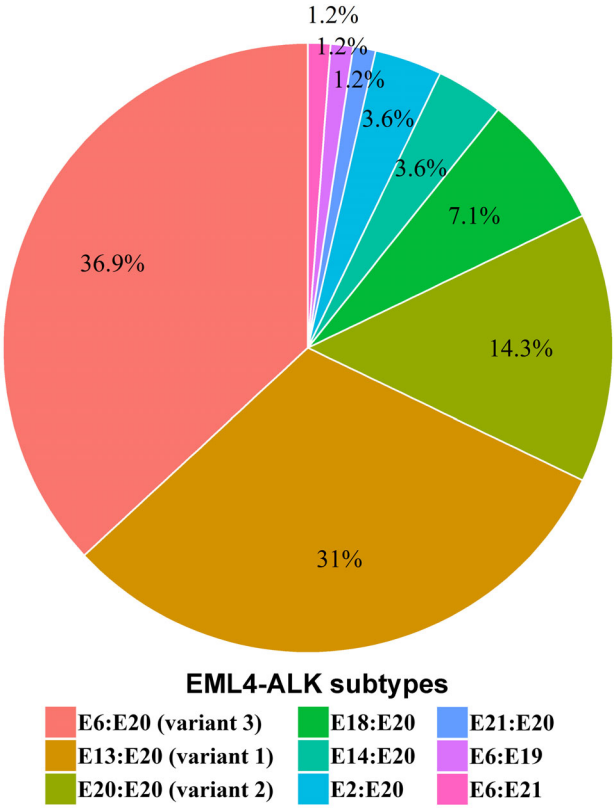

Figure 3. The frequency and distribution of EML4-ALK fusion subtypes identified in Chinese NSCLC cohort.

mutations and is associated with shorter survival in patients with lung cancer [58]. These results demonstrate that the patients with NSCLC with uncommon *EGFR* mutations exhibit a significantly inferior PFS compared with those with common *EGFR* mutations for first-generation TKIs. However, other studies revealed that the second-generation TKI afatinib was more active in patients with NSCLC who harbored certain types of uncommon *EGFR* mutations, especially G719, L861Q, and S768I [23], which indicated that accurate detection of uncommon *EGFR* mutations would help patients with NSCLC to select targeted therapeutic drugs more precisely. Besides, it was noteworthy that comutation of *EGFR* and *TP53* was frequently found in female patients in this study; another study showed that it was more likely to occur in young patients <45 years of age [57]. At the same time, the common mutation of *EGFR* and *TP53* is correlated with a poor prognosis [59]. It seems that young women should pay more attention to screening for NSCLC because their prognosis is poor in the event of NSCLC.

In addition to common druggable genomic alterations, frequencies of druggable tumor suppressor genes, which were considered uncommon genes in NSCLC, displayed a unique signature in Chinese populations. The incidences of *BRCA1/2* and *TSC1/2* were higher in the Chinese cohort than in the Western population. *BRCA1/2* were the well-known biomarkers for poly ADP-ribose polymerase (PARP) inhibitors, and *TSC1/2* naturally suppressed the overactivity of downstream mammalian target of rapamycin (mTOR), which indicated the potential clinical benefits of patients with *TSC1/2* loss of function mutations from mTOR inhibitors. However, compared with the Western population, the incidence of *CDKN2A/B* in Chinese patients with NSCLC was lower. Whether loss of function or deletion of *CDKN2A/B* related with clinical outcome of CDK inhibitors was not clear, but the lower incidence of these two cell cycle-related genes indicated the differences in pathogenesis in different ethnic groups.

In this Chinese cohort, pathogenic germline mutations were identified in a portion of younger patients, highlighting the necessity of risk assessment and management for those patients and their blood relatives. Two pathogenic or likely pathogenic germline mutations of *EGFR*, including a novel cancer susceptibility *EGFR* G724S mutation, were identified.

ALK rearrangement is often seen in NSCLC (3.1%), whereas its incidence in other tumors is only 0.2% [60]. The data in this study showed that the percentage of Chinese patients with *ALK* rearrangement was 7.8%, quite high compared with the published data mentioned above. Moreover, compared with

Table 5. Patient characteristics with uncommon EML4-ALK rearrangement

Patient	Histological subtype	Sex	Age, years	ALK rearrangement
P1	Lung adenocarcinoma	Female	55	EML4(exon6)-ALK(exon21)
P2	Lung adenocarcinoma	Male	45	EML4(exon6)-ALK(exon19)
P3	Lung adenocarcinoma	Male	40	EML4(exon21)-ALK(exon20)
P4	Lung adenocarcinoma	Male	52	EML4(exon2)-ALK(exon20)
P5	Lung squamous cell carcinoma	Male	53	EML4(exon2)-ALK(exon20)
P6	Lung adenocarcinoma	Male	53	EML4(exon2)-ALK(exon20)
P7	Lung adenocarcinoma	Male	59	EML4(exon18)-ALK(exon20)
P8	Lung adenocarcinoma	Male	69	EML4(exon18)-ALK(exon20)
P9	Lung adenocarcinoma	Female	45	EML4(exon18)-ALK(exon20)
P10	Lung adenocarcinoma	Female	75	EML4(exon18)-ALK(exon20)
P11	Lung adenocarcinoma	Female	51	EML4(exon18)-ALK(exon20)
P12	Lung adenocarcinoma	Male	54	EML4(exon18)-ALK(exon20)
P13	Lung adenocarcinoma	Female	40	EML4(exon14)-ALK(exon20)
P14	Lung adenocarcinoma	Female	49	EML4(exon14)-ALK(exon20)
P15	Lung adenocarcinoma	Male	46	EML4(exon14)-ALK(exon20)

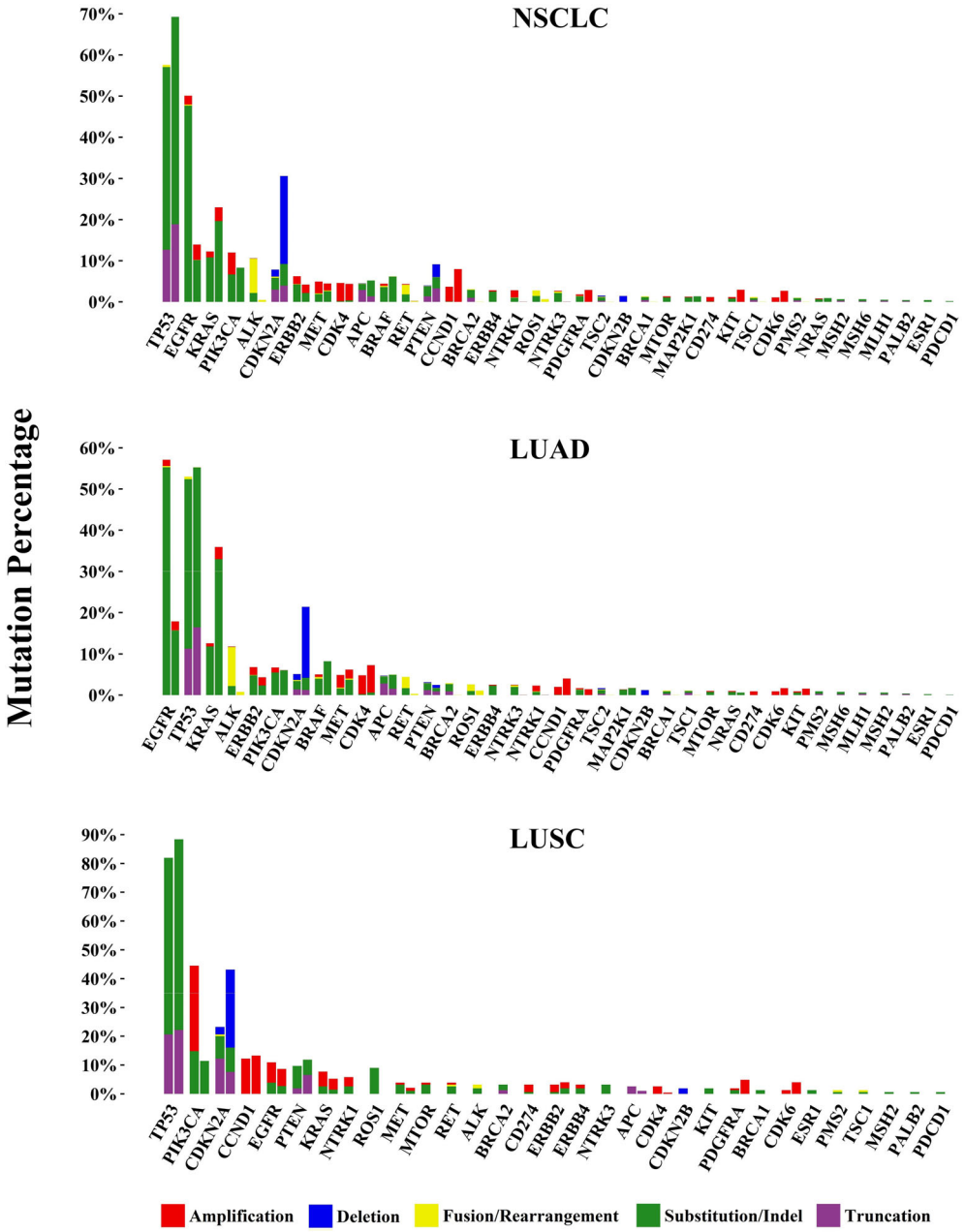

Figure 4. Genomic alterations in 1,200 cases of Chinese non-small cell lung carcinoma. 37 lung cancer-related genes and their composition of alteration types, including clinically relevant genomic alterations of the driver genes, are shown by cohort as a whole (NSCLC) as well as by adenocarcinoma cases (LUAD) and squamous carcinoma cases (LUSC). For comparison, corresponding The Cancer Genome Atlas figures are juxtaposed on the right.
Abbreviations: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer.

PCR-based *ALK* rearrangement assays in a similar cohort, the NGS-based assay identified more *ALK*-positive cases in NSCLC, suggesting that NGS was more sensitive than PCR for rearrangement detection. These data suggested that *ALK* rearrangement is more common in younger patients and that the relative frequency decreases along major age brackets, which is consistent with a previous report [31].

Based on different breakpoints in *EML4* and *ALK* genes, *EML4-ALK* can be divided into different subtypes [61, 62]. The most common subtypes were E13:A20 (1–13 exons in *EML4* were fused with 20–29 exons in *ALK*, variant 1), E20:A20 (variant 2), and E6a/b:A20 (variant 3a/b), which were detected in 31%, 14.3%, and 36.9% of patients with *EML4-ALK* NSCLC in the Chinese cohort, respectively. Clinical and preclinical studies have suggested differential clinical response to ALK inhibitors among different subtypes of *EML4-ALK.* For example, *EML4-ALK* variant 1 is associated with longer response duration to ALK inhibitors, whereas variant 3a/b is associated with earlier emergence of resistance to ALK inhibitors [61, 62]. Given that different variants respond differently to ALK inhibitors, the present findings may have important clinical implications. In the Chinese NSCLC population, the association between the incidence of *ALK* rearrangement and age was identified.

Table 6. Comparison of genomic alterations involving National Comprehensive Cancer Network guideline genes and KRAS between Chinese and Western non-small cell lung carcinoma

	SNV		Indel		Amp		Rearrangement		All[a]	
Gene	Percentage in Chinese cohort	Percentage cases identified by F1	Percentage in Chinese cohort	Percentage cases identified by F1	Percentage in Chinese cohort	Percentage cases identified by F1	Percentage in Chinese cohort	Percentage cases identified by F1	Percentage in Chinese cohort	Percentage cases identified by F1
EGFR	27	12	23	10	14	5.9	0.3	0	50	20
ALK	2.4	0.3	0	0	0.1	0.1	7.8	3.9	11	4.1
BRAF	3.4	5.1	0.2	0.06	0.6	0.4	0.3	0.2	4.4	5.7
ERBB2	2.2	0.8	2.2	2.6	2.4	3	0.1	0	6.3	6
MET	1.8	1.7	0	1.4	3	3.1	0.3	0	4.9	5.6
ROS1	1.4	0.1	0	0	0	0.03	1.3	1.3	2.8	1.5
RET	1.8	0.2	0	0	0.3	0.3	2.3	1.9	4.3	2.4
KRAS	11	31	0	0.04	2.1	3.5	0	0	12	32

[a]Some cases have multiple genomic alterations involving the same gene.
Abbreviations: Amp, amplification; EGFR, epidermal growth factor receptor; F1, Foundation One; indel, insertion and deletion; SNV, single nucleotide variant.

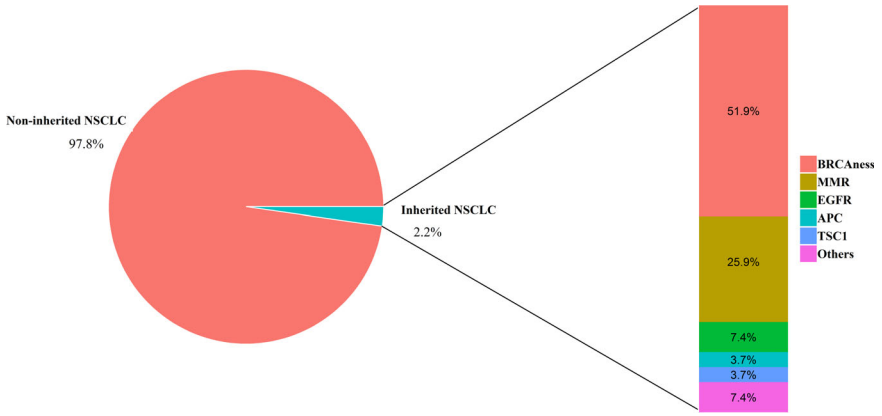

Figure 5. Germline mutations of cancer susceptibility genes identified with a multigene panel among 1,200 Chinese individuals with non-small cell lung carcinoma.
Abbreviations: EGFR, epidermal growth factor receptor; MMR, mismatch repair; NSCLC, non-small cell lung cancer.

Conclusion

In the present study, genomic signature of lung cancer-related driver genes identified by an NGS-based target sequencing assay showed higher frequency of uncommon *EGFR* mutations than traditional Sanger sequencing or ARMS, and higher prevalence of *ALK* rearrangements but fewer *KRAS* mutations compared with those in the Western population. In total, 73.9% of Chinese patients with NSCLC harbored at least one genomic alteration in druggable genes recommended by the NCCN guideline. These findings revealed that more patients would have the opportunity to benefit from current targeted therapies approved by the FDA. NGS-based targeted sequencing provided a more powerful tool to further fully understand the genomic features unique to Chinese patients.

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Disclosures

Aodi Wang: OrigiMed (E); **Hui Chen:** OrigiMed (E); **Peng Zhang:** OrigiMed (E); **Xiaowei Dong:** OrigiMed (E); **Yu-An Dong:** OrigiMed (E); **Kai Wang:** OrigiMed (E); **Ming Yao:** OrigiMed (E). The other authors indicated no financial relationships.

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